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Title: Seasonal variation in the relative importance of assembly processes in marine fish communities as determined by environmental DNA analyses

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Abstract (200 / 200 words)

Compositional variation among local communities is a result of environmental (e.g., environmental filtering) and spatial (e.g., dispersal limitation) processes. Growing evidence suggests that their relative importance varies temporally, but little is known about the short-time scale dynamics, that is, seasonality. Using marine fish communities in a Japanese bay as a model system, we tested the hypothesis that seasonal changes in the environment induce a shift in the relative importance of environmental and spatial processes. We used one-year monthly monitoring data obtained using environmental DNA and conducted a variation partitioning analysis to decompose the two processes. The relative importance of environmental and spatial processes was comparable averaged over the year but changed seasonally. During summer, when lower dissolved oxygen concentrations may adversely affect organisms, species composition was more explained by space despite larger environmental heterogeneity than in other seasons. This suggests that environmental processes weakened during the season with extremely severe environments, likely due to the random loss of individuals. We conclude that the assembly processes of communities of mobile organisms, such as fishes, can shift even within a year in response to seasonal changes in environmental severity. The results also indicate the applicability of eDNA techniques for community assembly studies.

Keywords:

Community assembly, dispersal limitation, eDNA, environmental filtering, fish, temporal assembly

Background

Understanding the processes structuring local communities has been a central challenge in ecology (Hutchinson 1959, HilleRisLambers et al. 2012). Local communities are structured by the interaction of two types of assembly processes (Cottenie 2005, Mouillot 2007, Chase and Myers 2011). Environmental processes affect species composition in a deterministic manner, such that the local environment sorts species that cannot colonise and persist in a given habitat. Conversely, spatial processes, such as dispersal limitation and random migration, can influence the local community structure in a more stochastic manner, irrespective of species properties (Sale 1978, Hubbell 2001). With the increasing acknowledgement that both processes act in concert, much research has been devoted to understanding their relative importance (Cottenie 2005, Chase and Myers 2011, Ford and Robert 2018). Previous studies have revealed the changes in the importance of spatial and environmental processes over time. Generally, spatial processes are more relevant during earlier phases of community assembly, whereas environmental processes occur during later phases because of the time lag of species colonisation (Allen et al. 2011, Alexander et al. 2012, Helsen et al. 2013). These insights have suggested that natural communities are not at equilibrium but instead are in the transient course of approaching it, and are key to a holistic understanding of the mechanisms that shape community structures over long temporal scales (Chang and HilleRisLambers 2016), from years (Helsen et al. 2013) to decades (Allen et al. 2011, Alexander et al. 2012).

Despite the growing evidence that community assembly processes vary temporally, little attention has been given to short temporal scales such as seasonal dynamics (Holyoak et al. 2020), probably reflecting the biased focus of previous studies on organisms with low

mobile ability, such as plants (Alexander et al. 2012, Helsen et al. 2013) or microorganisms in closed water systems (Allen et al. 2011). As mobile organisms can move between habitats quickly in response to environmental changes, the relative importance of environmental and spatial processes can vary even within a year. For example, a limited number of studies have suggested that the interspecific interaction among riverine fish species is stronger in the dry season when individuals are densely concentrated in limited habitual areas (Fitzgerald et al. 2017). In contrast, Mouchet et al. (2013) showed that fish assemblages in a lagoon is constantly regulated by the environment throughout a year. Therefore, whether the seasonal variation of assembly processes is a commonly observed pattern remains an open question. Short-time scale variation in the relative importance of spatial and environmental processes, in contrast to the long-time scale variation, would imply that the assembly processes temporally fluctuate irrespective of the equilibrium states.

In addition, there are contrasting predictions regarding the consequences of seasonal changes in the environment. During the season with harsh conditions, the environment is expected to have a stronger effect on the species composition (Chase 2007, Guo et al. 2014). However, it is also probable that an extremely severe environment can induce extinction or recolonisation of individuals irrespective of species traits, negating the effect of environmental processes (Lepori and Malmqvist 2009). In particular, knowledge is scarce in open systems such as the ocean, where the movement of individuals is less limited and can be more responsive to environmental changes (Travers et al. 2006, Kopp et al. 2012). Furthermore, as marine ecosystems are structured both vertically and horizontally (Travers et al. 2006, McClain and Rex 2015), looking into the environmental gradient along

water depth would be essential for assessing fish community assembly. The knowledge gap in marine ecosystems partly stems from the considerable effort required to assess marine fish species composition at multiple sites, multiple water depths, and continual time points within a year (Yamamoto et al. 2017).

This difficulty can be substantially overcome by environmental DNA (eDNA) analysis, which has emerged as a powerful and efficient tool for monitoring biodiversity, especially in aquatic ecosystems (Bohmann et al. 2014; Deiner et al. 2017; Lacoursière-Roussel et al. 2018). By extracting genetic materials shed from organisms from environmental samples (e.g., water and soils), this technique allows non-invasive assessment of biodiversity. Its efficiency in detecting species has been illustrated by studies showing that the number of marine fish species detected by eDNA analysis was comparable to or larger than that detected by traditional sampling methods, such as visual census (Thomsen et al. 2012; Sigsgaard et al. 2017; Yamamoto et al. 2017). Furthermore, owing to the short persistence (from a few hours [Murakami et al. 2019] to several days [Thomsen et al. 2012]) and low diffusion rate (less than 100 m; Port et al. 2016; Murakami et al. 2019) of eDNA in seawater, this technique enables the estimation of contemporary and local species composition. Taking advantage of this technique has the potential to help evaluate the relative importance of environmental and spatial processes in a whole fish community, but such applicability has not been tested so far.

The objectives of this study were (1) to determine whether the relative importance of environmental and spatial processes of marine fish community assembly changes seasonally and (2) to explore how the severity of the environment affects their relative importance. This study was conducted in Tokyo Bay, one of the largest bay areas in Japan.

113 Its environment is known to be subject to large seasonal changes. In particular, lower
114 concentrations of dissolved oxygen in summer induced by stratification of water columns
115 (Yasui et al. 2016) can serve as a severe environment for fish species, especially in the
116 bottom water (Kodama and Horiguchi 2011). To test the hypothesis that seasonal changes
117 in environmental severity induce a shift in the relative importance of environmental and
118 spatial processes, we used one-year monthly monitoring data on fish community
119 assemblages at 14 sites in Tokyo Bay, which were obtained by eDNA analyses of samples
120 collected from both surface and bottom waters. The variation partitioning method was
121 applied to decompose the variation in species composition into those explained by
122 environmental or spatial variables.
123

Materials and methods

Water sampling and eDNA sequencing

Water samples were collected monthly from 14 sites in Tokyo Bay, Japan (approximately 1380 km^2, 35°30'N, 139°50E, Fig. S1) from January to December 2019. At each sampling, 1 L of water each was collected the surface and bottom waters. Bottom depth varied among sites from approximately 6 to 70 m. Water samples were filtered using a GF/F membrane (0.7 μm pore size; Cytiva, Sheffield, UK) per litre, and the filters were frozen on board a ship. The frozen filters were then transferred to and stored in the laboratory. The extraction of eDNA was performed as described by Yamamoto et al. (2016).

Water temperature, salinity, and dissolved oxygen (DO) were measured at the same time as water sampling. We also measured four additional variables (chlorophyll-a concentration, turbidity, conductivity, and water density), but they contained numerous missing values because of some logistic problems and thus were not used in the analyses. To examine whether the three main variables well represented the overall environment, we calculated Spearman's rank correlation coefficient for each pair of the full (seven) variables from a limited number of samples. The correlation analysis indicated that the environmental variables that were not included in the main analyses were highly correlated with at least one of the three main variables (water temperature, salinity, and DO) (Spearman's rank correlation coefficient > 0.5 or < -0.5, Table S1).

For the eDNA samples, we employed a two-step polymerase chain reaction approach to amplify the mitochondrial 12S rRNA gene using MiFish universal primers developed by Miya et al. (2015). The detailed procedure is provided in Hongo et al.

147 (submitted). The constructed amplicon sequencing variants (ASVs) were assigned to fish
148 species using Blastn against the MitoFish database (Sato et al. 2018). To keep the dataset
149 free from unexpected biases such as sequencing errors or contamination from nearby fish
150 markets, some of the species that did not meet the following criteria were filtered out from
151 the further analyses: (1) those whose names were not matched during the search in Fishbase,
152 a global database of fish (Froese and Pauly 2019; https://www.fishbase.se/), and (2) those
153 whose habitats were known to be out of Tokyo Bay based on information from several
154 literatures (Nakabo 2002, Kouno et al. 2011). The first criterion mainly filtered out hybrid
155 species, while the second one, which was based on each fish species' distribution in Japan
156 and observation records in Tokyo Bay, removed fish species known to be endemic in other
157 regions or countries.

158 We obtained 308 water samples, and eDNA extraction and gene amplification
159 were successful for 281 samples (91%). Among these, corresponding environmental data
160 were available for 225 samples (n = 22, 22, 22, 21, 20, 20, 18, 21, 20, 2, 15, and 22 from
161 January to December, respectively), which were submitted to the variation partitioning
162 analysis described below. A total of 220 fish species were detected in 225 samples. Of
163 these, our first criterion filtered out 12 species, and the second criterion eliminated 40
164 species, resulting in 168 species retained in the subsequent analyses (Table S2).

165

166 *Statistical analyses*

167 All statistical analyses were performed using R version 4.0.2 (R Core Team 2020).
168 First, to capture the seasonal trend of the environment, we conducted a principal component
169 analysis (PCA) on the three environmental variables (water temperature, salinity, and DO).

To visualise the seasonal changes in the species composition, we conducted nonmetric multidimensional scaling (NMDS) on the whole species composition data (n=281) using the *metaMDS* function in the *vegan* package (Oksanen et al. 2019). This procedure allows the compositional dissimilarity among data to be interpreted in a low-dimensional space by positioning similar data nearby. For the NMDS analysis, we used the Jaccard dissimilarity index on the presence-absence dataset and three dimensions (k=3) to facilitate convergence. Confirming that using the Sorensen dissimilarity index did not qualitatively alter the result, we illustrated the results obtained by using the Jaccard dissimilarity index. Because our hypothesis focused on the consequences of the severe environment in summer, especially in the bottom water, we tested the difference in the species composition among the bottom and surface waters for each month separately, using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) implemented by the *adonis* function with 9,999 permutations.

The relative importance of environmental and spatial processes was evaluated using the variation partitioning method (Borcard et al. 1992). Specifically, we conducted a series of distance-based redundancy analyses (db-RDA, Legendre and Anderson 1999) separately for each sampling month. This multivariate analysis takes the dissimilarity (Jaccard index based on presence-absence data) matrix of species composition among samples as a response variable and three types of variables (environment, horizontal space, and vertical space) as explanatory variables. The variables of horizontal and vertical space are referred to as “space” and “water depth”, respectively, for brevity hereafter. Conducting a series of db-RDAs with different combinations of these three explanatory variables allows the variation of the response variable to be partitioned into several components (Borcard et

al. 1992; Fig. 1), that is, (1) the unique contribution of environmental ([a] in Fig. 1), spatial ([b]), and water depth variables ([c]); and (2) the combined effect of each pair of the three variables ([d], [e], [f]), or that of the three variables ([g]). The unique contribution of environmental variables reflects strong environmental filtering, whereas that of spatial and water depth variables suggests that species composition is spatially structured irrespective of the environment (Borcard et al. 1992). The combined effects of the variables indicate that the explanatory variables are correlated (e.g., the environment is spatially structured), and thus the contribution of each variable is inseparable.

Environmental variables included normally scaled (mean = 0, SD = 1) values of the three environmental variables (water temperature, salinity, and DO). Spatial variables were obtained using principal coordinates of neighbour matrices (PCNM; Borcard et al. 2004) which extract the spatial pattern of multiple scales from the coordinates of the sampling sites. From this, eight PCNM axes with positive eigenvalues were created using the *pcnm* function with a threshold of 11,323 m (Fig. S1). As for water depth, we used the identity of the depth of the water sampling (i.e. surface or bottom). We conducted complementary analyses using the absolute value of the water depth, confirming that the main results were qualitatively similar. To maintain consistency with the other analysis (PERMANOVA), we show the results obtained using the identity of the water depth as an explanatory variable in the main text. To enable the comparison of the results of the variation partitioning analysis among different sampling months, we avoided performing variable selection before variation partitioning. However, regarding the spatial variable, including all the PCNM values is known to result in the overestimation of their explanatory power by capturing quasi-random spatial variation (Gilbert and Bennett 2010), and we did

note such observations in our dataset (Supplemental result, Fig. S2). Therefore, we conducted forward selection separately for the spatial variables based on their *P*-values for each season (eight PCNM values) following Blanchet et al. (2008) using the *capscale* and *ordistep* functions in the *vegan* package. The environmental variables, water depth, and selected spatial variables were subjected to variation partitioning analysis using the *varpart* function. Due to the small number of sampling sites (n = 2) because of logistical problems, we did not conduct a variation partitioning analysis for the October data.

Results

The PCA of the three environmental variables clarified their seasonal trends (Fig. 2). The first two PC axes explained 90.8% of the total variance. The first axis positively correlated with salinity (r = 0.935) and negatively correlated with temperature (r = −0.703) and DO (r = −0.443), and the second axis positively correlated with DO (r = 0.859) and negatively correlated with temperature (r = −0.645). Low concentrations of DO and differences between the surface and bottom waters were apparent from June to September. Parallel with this, water salinity diverged between samples such that the surface water was characterised by lower salinity, which is likely a result of the rainy season in June. Due to this divergence in the environment, the overall heterogeneity of the environment was apparently high from June to September, compared to that in the other months.

The NMDS illustrated the seasonal changes in species composition (Fig.3). Throughout the study period, species composition was significantly different between the surface and bottom waters in most months (P < 0.05, PERMANOVA, statistics are shown in Fig. 3), except for October and November. However, we observed an abrupt decrease in the R^2 value in June, suggesting that the surface-bottom separation of species composition was weaker during summer and post-summer (June to December).

The variation partitioning analysis based on db-RDA explained a moderate proportion of the variation in species composition (19.3% of the total variance, averaged for months, Fig. 4a). Overall, the environment, space, and water depth contributed to a similar degree of variation (11.5%, 10.2%, and 7.9%, respectively, averaged over months).

245 However, the effect of each variable showed a seasonal variation, particularly during June
246 and July. Some of the contribution of environmental variables was confounded with that of
247 water depth from March to September, but that component almost disappeared in June and
248 July (Fig. 4b). In contrast, the pure contribution of spatial variables was larger in June and
249 July than that in the other months (Fig. 4c). Regarding the proportion explained by water
250 depth, its contribution was low during June and July, as well as during November and
251 December.
252

Discussion

In this study, we tested the hypothesis that the relative importance of environmental and spatial processes of marine fish community assembly changes seasonally in response to the shift in the environmental severity. Overall, species composition was explained by environmental and spatial variables to a similar extent, coinciding with previous evidence of environmental filtering (Mouchet et al. 2013, Pecuchet et al. 2016) and stochastic assembly (Sale 1978, Ford and Roberts 2018) in marine fish communities. However, during summer, when a lower concentration of oxygen serves as a severe environment for the organisms, the pure contribution of spatial variables was large, suggesting that fish species composition was spatially structured irrespective of environmental heterogeneity.

The increase in the pure contribution of spatial processes in June and July reflects the discrepancy in the timing of the divergence of the environment and species composition. Although fish species composition was dissimilar between the surface and bottom waters throughout the year, the differences were less evident in summer and the subsequent season, as indicated by lower R^2 values (Fig. 3). Conversely, the environment started to diverge between the surface and bottom waters around May and June (Fig. 2), likely because of the stratification of water columns, which induces lower DO in the bottom water (Yasui et al. 2016), and the rainy season in June, which decreases the salinity of the surface water. This indicates that, during summer, fish species composition was more similar between the surface and bottom waters despite the higher heterogeneity of the environment, which should have resulted in a weaker explanatory power of the environmental variables (Fig.4b). As the surface and bottom waters were sampled at the same sites sharing the same coordinates, the compositional similarity between the surface and bottom waters was

276 detected as the apparent effect of spatial variables in the variation partitioning analysis
277 (Fig.4c). In contrast, from March to May, the species composition was clearly divided
278 between the surface and bottom waters (Fig. 3). As the surface–bottom distinctiveness in
279 the environment was also evident during that period (Fig. 2), variation partitioning
280 indicated that the importance of the environment was structured in the depth direction (Fig.
281 4b).

282 The results indicate that environmental filtering is less important during the
283 summer when DO is limited. Contrary to our findings, some previous studies have shown
284 that environmental filtering is stronger in harsher environments in terms of abiotic stress
285 (Chase 2007, Guo et al. 2014), productivity (Chase 2010), and disturbance (Lepori and
286 Malmqvist 2009) because of the reduced probability of ecological drift and alternative
287 stable states. However, others have suggested the possibility of a more stochastic assembly
288 (i.e. weaker environmental filtering) in harsher environments, because extreme
289 environments can induce death of individuals irrespective of species identity (Lepori and
290 Malmqvist 2009) or because species that survive very stressful conditions may share
291 similar traits and be nearly neutral (Kim et al. 2019). In the present study, we argue that the
292 former mechanism of species-independent loss by environmental harshness is likely due to
293 the following evidence on the number and occurrence probabilities of species detected: the
294 number of detected species decreased in the bottom water and increased in the surface
295 water from June and July (Fig. S3), suggesting that individuals in the bottom water were
296 forced to move toward the surface (Pihl et al. 1991) or perhaps experienced higher
297 mortality due to the depletion of oxygen (Kodama and Horiguchi 2011). Furthermore,
298 given that the species composition became less distinctive in June and July (Fig. 3), it is

299 likely that such movement or extinction occurred without deterministic selection,
300 irrespective of species identity. Supporting this, for 13 out of the 16 most common species
301 (the top 10% in our dataset), the relative occurrence probability in the bottom versus that in
302 the surface waters was lower during June and July than that in other months. For example,
303 *Lateolabrax japonicus* (Japanese sea bass), the third most common species, was detected in
304 48.7% of the surface water samples and 71.1% of the bottom water samples during the
305 study period, except for June and July, suggesting a preference for the bottom environment.
306 However, in June and July, when the bottom environment was severe, the occurrence
307 probability was even lower at the bottom (53.8%) than that at the surface (56.2%). These
308 results collectively suggest that the limited oxygen in the bottom water during June and
309 July adversely affects the performance of fish individuals, irrespective of their species
310 identity. Importantly, the seasonal shift in the importance of environmental filtering could
311 be detected only by comparing the environment and species composition between the
312 surface and bottom waters. Indeed, a previous study which did not consider the
313 bottom-surface distinction showed no seasonal trend in the strength of environmental
314 filtering (Mouchet et al. 2013). Therefore, to examine the seasonal changes in the assembly
315 processes of marine fish communities that are inherently structured both vertically and
316 horizontally, incorporating the vertical dimension into community assembly studies is
317 essential.

318 Our finding that the assembly processes of marine fish communities changed
319 seasonally challenges the perspective that natural systems are at equilibrium. The
320 equilibrium assumption can be seen in previous studies that disentangled the assembly
321 processes based on snapshot data collected at one time. Although such studies have

provided pivotal insights into the mechanisms determining species composition, increasing evidence shows that the relative importance of environmental and spatial processes varies temporally (Allen et al. 2011, Alexander et al. 2012), suggesting that inferences from snapshot data would be insufficient for a holistic understanding of the mechanisms that shape community structures across a long temporal scale (Chang and HilleRisLambers 2016). More importantly, we revealed a seasonal shift in the assembly processes, which is a much shorter time scale than previously investigated (several years to several decades; but see Fitzgerald et al. 2017). Previous findings suggested that environmental processes replace spatial ones as the assembly progresses, postulating that communities approach equilibrium in the long run (Allen et al. 2011, Alexander et al. 2012). In contrast, our results provide an alternative view that the relative importance of the two processes can temporally fluctuate without any direction. This may be particularly true for marine fish communities, because fishes have high mobility, and thus their distribution and species composition can change quickly in response to seasonal environmental changes (Travers et al. 2006, Kopp et al. 2012). Therefore, when studying the community assembly processes of mobile organisms such as fish, more insights may be gained by incorporating two types of temporal perspective, that is, toward-equilibrium dynamics that occur over a long-time scale and non-equilibrium fluctuations that occur in a short-time scale. When studying community assembly processes from snapshot data, care should be taken because analysing data from a single season may over- or underestimate the significance of the environmental and spatial processes.

Although the ability of eDNA to detect species from environmental samples has been well examined (Thomsen et al. 2012; Sigsgaard et al. 2017; Yamamoto et al. 2017),

this technique has rarely been applied to the evaluation of community assembly processes. Our results indicate that eDNA analysis can clearly reveal the relative importance of environmental and spatial processes in fish community assembly, in combination with the availability of environmental variables. However, most of the variation (nearly 80%) in the species composition remained unexplained by environmental, spatial, and water depth variables (Fig. 4), although a comparable level of low explanatory power is common in these kinds of analyses (e.g., 13%–21% for marine fish communities [Ford and Robert 2018] and 36.7% for forest communities [Borcard et al. 1992]). We suspect that the main reason for this may be that the dataset we used contained only presence–absence information. Estimating species abundance data from eDNA samples is challenging because the correlation between eDNA concentration and abundance of target fish species is variable under natural conditions (Yates et al. 2019). To avoid potentially biased results, we did not use the read number of DNA as an abundance index, which might result in a loss of information. Furthermore, since the detection of eDNA is influenced not only by the presence of the species but also by the degradation and accumulation of the eDNA itself ("ecology of eDNA" sensu Barnes and Turner 2016), our results may have been subject to such biases. Previous studies have shown that the degradation of eDNA after release into water is faster at higher temperatures (Tsuji et al. 2017, Jo et al. 2019), and it has been assumed that eDNA accumulates in surface waters (Eichmiller et al. 2014, Moyer et al. 2014). These may be a source of bias in our results by affecting the detection probability of the species. However, we consider that this was not the case here or at least not so influential, because the number of detected species was higher in summer (when

367 degradation is expected to be faster due to higher temperatures) and in samples from the
368 bottom (where accumulation is expected to be reduced) (Fig. S3).
369

Conclusion

In ecosystems, species assemblages are the result of a combination of environmental and spatial assembly processes. We found that, in marine fish communities, their relative importance changes seasonally, such that vertically structured environmental gradients became less influential during summer, the season characterised by a lower concentration of DO. This result suggests that the effect of environmental filtering is weak in an extremely severe environment, likely due to the random loss of individuals. We conclude that marine fish communities are dynamic and, therefore, it would be a fruitful approach to incorporate short-scale temporal perspectives in studying their assembly processes. Furthermore, by applying the eDNA analyses for evaluating community assembly processes for the first time to our best knowledge, this study highlights the potential applications of this promising technique across a wide range of disciplines of community ecology.

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387

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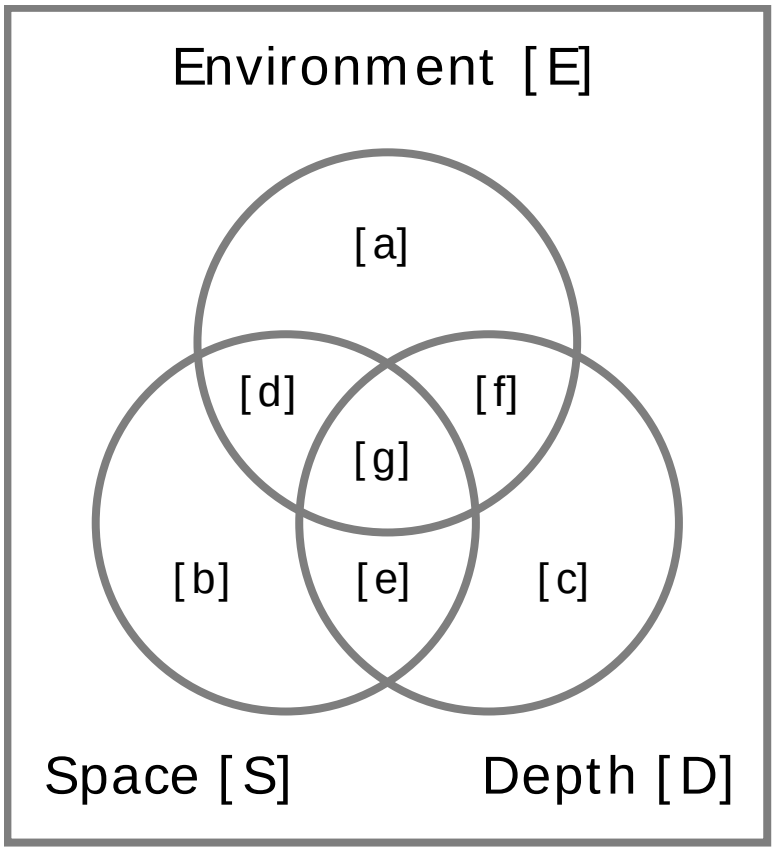
Figures and tables

Figure 1 Schematic representation of the inference of community assembly processes using the variation partitioning approach. Variation in the species composition is decomposed into components explained by environmental and spatial variables, and water depth: [a-c] Pure contribution of each of the variables. [d-f] Combined contribution of each pair of the three variables and [g] that of the three variables, which indicates that the variables are correlated (e.g., the environment is spatially structured) and thus their contribution is inseparable.



552 Figure 2 Principal component analysis of three environmental variables (water
553 temperature [Temp], salinity [Sal], and dissolved oxygen [DO]) illustrated
554 separately for each month. Arrows in the top-left panel indicate the three
555 original environmental variables. Point colour represents the difference in the
556 water depth of the sampling (red: surface, blue: bottom). Note that the number
557 of data is smaller than that of species composition (Fig. 3) because of some
558 logistic problems, especially for October.

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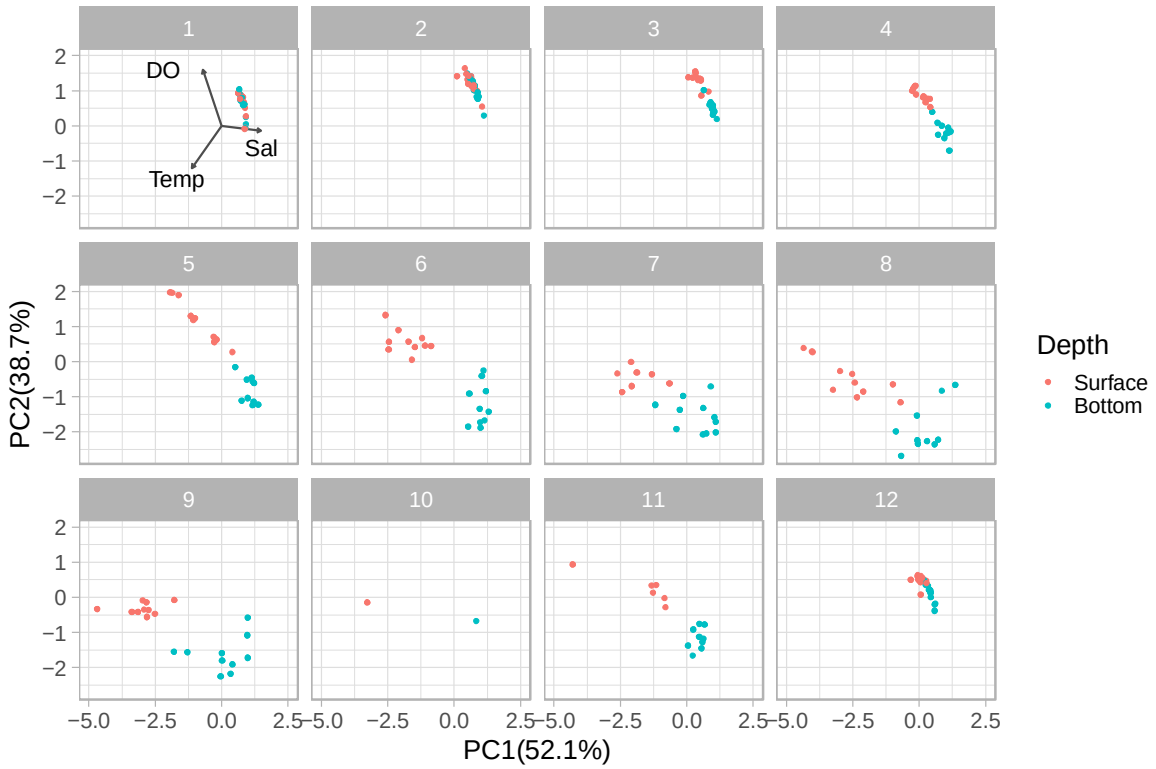

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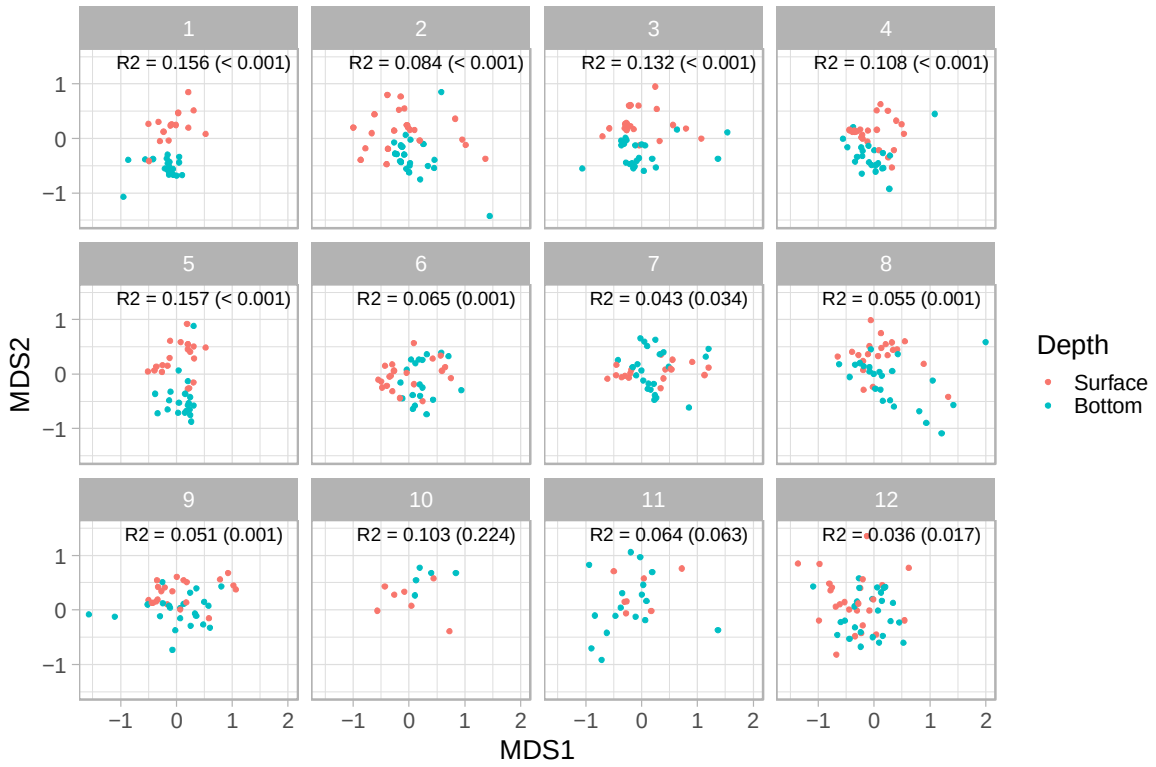
Figure 3 Nonmetric multidimensional scaling (NMDS) results for species composition based on Jaccard dissimilarity. Point colour represents the difference in the water depth of the sampling (red: surface, blue: bottom). Values in each panel are the statistics from the permutational multivariate analysis of variance that tested the difference in species composition between the surface and bottom waters (R^2 value is shown with *P*-value in parentheses). Stress value of the NMDS is 0.193.



572 Figure 4 (a) Variation partitioning of species composition into components explained by
573 environment (E), space (S), water depth (D), and their combinations. Each of
574 the pure and combined components (see Fig. 1) are illustrated with different
575 colours. (b-d) Same results of the variation partitioning, but the components are
576 illustrated separately for environment, space, and depth for visualization
577 purposes. The analysis was not conducted for the October data because of the
578 small number of data (n = 2).
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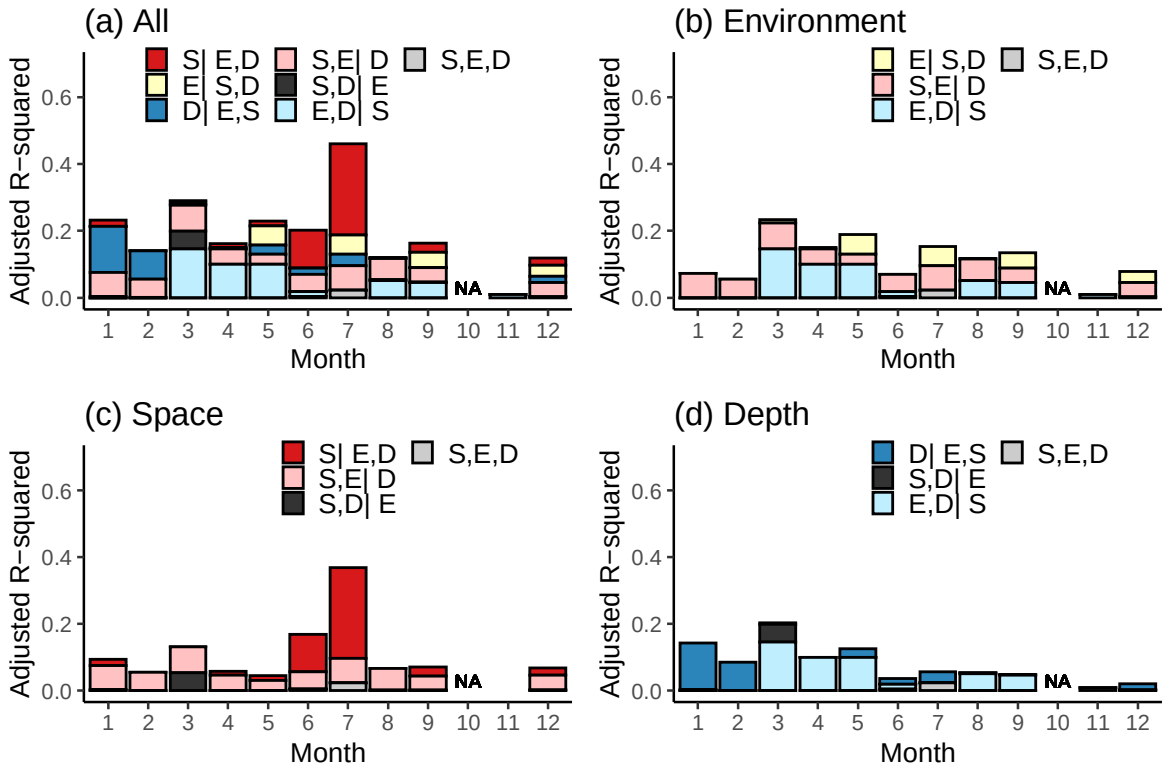

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